TAXONOMIC AND CYTOLOGICAL STUDIES ON THE CILIATES ASSOCIATED WITH THE AMPHIPOD FAMILY ORCHESTIIDÆ FROM THE WOODS HOLE DISTRICT

I. The Stomatous Holotrichous Ectocommensals

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This is the first of a series of studies undertaken in an effort to contribute to our knowledge of the ciliates, both commensal and parasitic, that are associated with various invertebrate hosts in the region of Woods Hole, Massachusetts. The present paper deals with the stomatous ectocommensals of three species of the family Orchestiidæ: *Talorchestia longicornis* (Say), *Orchestia agilis* Smith, and *Orchestia palustris* Smith. The study includes not only the morphological details necessary for the determination of the taxonomic rank of this group of ciliates but also a cytological study of the process of binary fission of one of the species.

The material for this investigation was collected in the vicinity of Woods Hole, Massachusetts and the studies carried out at the Marine Biological Laboratory. We wish to express our appreciation to Miss Florence Stuck of Columbia University for calling our attention to the occurrence of some of the ciliates here described.

Material and Methods

Our initial material was collected during the summer of 1931 from Nobska Beach. At this time a number of specimens of the sand flea *Talorchestia longicornis* were taken and transferred in moist sand to New York City where a preliminary study of the ectocommensals was made. During the summer of 1934 intensive collections of the same species of amphipod together with *Orchestia agilis* and *Orchestia palustris* gave us ample opportunity to continue our observations. *Orchestia agilis* and *Orchestia palustris* were also collected from Sippewisset Beach and from the muddy banks of the Eel Pond in Woods Hole.

Talorchestia is found buried in the sands during the day near the high water mark. It is a relatively simple matter to obtain abundant material by digging them out and placing them in a container partially filled with moist sand. *Orchestia agilis* is found in great abundance

among the moist and decaying sea weed that has been beached by the tide. They can be collected by the thousands by shaking some of this sea weed over any vessel, care being taken to cover the vessel quickly to prevent their escape. *Orchestia palustris* burrows in the mud under decaying vegetation and must be collected individually. As this species is much less active than the preceding one, abundant material can be collected in a minimum of time.

In all cases the amphipods were brought directly to the laboratory where the preparations of their ectocommensals were made. As the material was abundant, ample opportunity was afforded for a detailed study in the living condition of all of the species of ciliates here described. For the cytological details permanent preparations were made by crushing the host on an albuminized cover glass, the ciliates coming away from the carapace in the body fluids. These smears were then fixed and stained in any desired manner.

For morphological observation the best results were obtained by fixing the ciliates in strong Flemming's fluid, staining in Heidenhain's hæmatoxylin and differentiating in a 10 per cent solution of superoxol, following the method described by Kidder (1934*a*). Excellent results were also obtained by the use of the Borrel mixture following hot (60° C.) Schaudinn's fluid.

For the details of the divisional activity the Feulgen nucleic-acid reaction following hot (60° C.) Schaudinn's fluid proved invaluable, although each stage was checked with material stained in Heidenhain's hæmatoxylin and differentiated in iron alum.

Taxonomy

Only two genera of holotrichous ciliates are found to be associated as commensals with the three species of amphipod hosts studied. The most abundant ciliates belong to a hitherto undescribed genus, which we have named *Allosphærium* gen. nov., of which there are five distinct species. Less abundant, but occurring regularly, are found three undescribed species of the genus *Chilodonella* Strand.

Allosphærium gen. nov.

Allosphærium is a small (24 μ to 59 μ) holotrichous ciliate. All of the species so far discovered live as commensals on the carapace and gill lamellæ of three species of amphipods (*Talorchestia longicornis*, *Orchestia agilis*, and *Orchestia palustris*). They are remarkably adapted for this mode of life, being very flat dorso-ventrally and possessing great thigmotactic powers. When washed onto a slide and studied in life it is seen that they adhere tenaciously to the glass or to the surface film of

the water. They creep rapidly along these surfaces with a rather steady motion moving in wide circles to the left. When dislodged they swim rapidly in a jerky fashion and quickly settle to any surface with which they come in contact.

When viewed from the dorsal surface these ciliates are nearly oval, although slightly asymmetrical. The left lateral margin conforms more nearly to the anterio-posterior axis than does the right, which is curved in a wide arc. The anterior end is usually slightly pointed while the posterior end is evenly rounded in most cases, although in some it is nearly truncate.

The dorsal surface is arched and devoid of cilia while the ventral surface is slightly concave and covered with from twelve to twenty-seven rows of fine cilia. On the ventral surface the lateral pellicle is extended into two folds partially enclosing the concave ciliated area. The right fold is smooth and extends nearly or wholly the length of the body, while the left fold is more in the form of a lapel, smooth in some cases, notched in others.

The cytostome is located near the anterior end on the ventral surface of the body. It is oval or irregular in shape depending on the species. The posterior border of the cytostome is guarded by a shelf or ridge, perpendicular to the long axis of the body and extending to the left lateral margin. Anterior to this ridge the ventral surface falls away into a trough which forms a naked, rather shallow oral groove. The cytostome is equipped with peculiar specialized ciliary elements. Originating within the oral opening and extending well to the outside are three groups of fused cilia, forming three separate membranes. These membranes are pointed at their distal ends and fan-shaped proximally. Two of them originate from the posterior wall of the cytostome and somewhat overlap one another while the third is always more narrow and originates from the right wall of the cytostome. The ones posterior beat out and up in a direction parallel to the long axis of the body. The narrow lateral membrane beats out and toward the left in a direction perpendicular to the long axis of the ciliate. The cilia of the membranes are fused and beat synchronously as a single unit but the membranes are not synchronized with each other. Their movements are discontinuous and their function is presumably to sweep food into the mouth in much the same manner as the pseudomembranelles of *Kidderia* Raabe 1934*a* (*Conchophthirius*) *mytili* (Kidder, 1933*a*).

There is a single macronucleus, more or less oval in shape, located near the center or slightly anterior to the center of the cell. The single micronucleus is situated just anterior to the macronucleus.

In four of the five species of this genus there are two well-developed contractile vacuoles, one in the anterior fourth of the body well toward the left margin, and one in the posterior fourth of the body, either centrally located or toward the left margin of the cell. The fifth species possesses but one contractile vacuole which is situated toward the left lateral edge just anterior to the cell center.

In the posterior cytoplasm well toward the left side of the body is found a curious inclusion which is constant for all species of the genus. In life this inclusion is in the form of a refractile sphere while in hæmatoxylin preparations it stains intensely. It decolorizes more readily than do the nuclei but can easily be identified, especially in Flemming-Heidenhain preparations. It is negative to the Feulgen nucleic-acid reaction and does not stain with either neutral red or Janus green. The acid (green) component of the Borrel mixture stains it with great intensity. All fixations used, whether or not they contain acetic acid, preserve it. In life as well as in stained preparations this endoplasmic sphere always appears homogeneous as to structure and constant as to shape. Our observations to date have given us no clue as to the possible significance or function of this unique cell inclusion. Because of its behavior during cell division, as will be noted later in this report, we are inclined to regard it as a metaplastid, but one that is very regular in its appearance within the species of this genus.

The five species of the genus *Allosphærium* form a closely integrated group. They differ from one another in average sizes although there is considerable overlapping at the extremes. The number of rows of cilia on the ventral surface is constant for a given species and this criterion, taken with the differences in shape, size, and appearance of the nuclei as well as the ventral pellicular folds, makes it possible to differentiate one species from another readily.

There appears to be little host specificity in this group as all three species of amphipods used in this investigation were found to carry an infestation of at least two of the species of *Allosphærium*.

Following is a short general description of the five species of the genus *Allosphærium*.

Allosphærium palustris gen. nov., sp. nov. (Fig. 1, *A*).—This ciliate is the largest species of the genus so far encountered. It averages 55 μ in length (46 μ to 59 μ). The dorsal surface is weakly arched and possesses three folds. One fold occurs along the right margin in the posterior half. It follows the general contour of the body and terminates near the posterior end of the ciliate. The other two start just posterior to the level of the cytostome near the left margin and end at about the level of the endoplasmic sphere. These dorsal furrows are constant in

this species. They disappear only after the ciliate becomes flattened, between the slide and cover, to the extent that only feeble motion is possible.

The ventral surface is nearly flat and covered with twenty-seven rows of fine cilia. Of these twenty-seven rows three originate anterior and to the left of the cytostome, curve around its anterior extremity and follow the contour of the right lateral margin of the body and finally end in an oblique suture near the left posterior edge of the ciliate. The remaining twenty-four rows originate in a line just posterior to the cytostome and proceed backwards. Half of them swing down in an arc to enter the suture from its posterior right side, while the other half either converge at the innermost tip of the suture or enter it from its left anterior side. The cilia in the mid-region of the body are rather short while those of the anterior portion about the mouth and those of the extreme posterior region of the cell are quite long. All of these peripheral cilia beat metachronously.

The cytostome is relatively large, the outlines being regularly notched on the right side. The two posterior ciliary membranes are large and triangular while the right membrane is narrow and long.

The right ventral pellicular fold is very narrow and shallow, extending the full length of the body. The left fold starts just anterior to the center of the cell and is extremely broad. It is smoothly rounded and overlaps a considerable portion of the left posterior region of the ventral surface.

The endoplasmic sphere is always situated just at the inner end of the oblique suture. It is relatively small in comparison with the same inclusion of some of the other species of this genus.

There are two contractile vacuoles, one located just anterior and slightly to the left of the endoplasmic sphere and the other situated near the right border of the cell in the anterior fifth of the body. Their diastolic period is quite long and there appears to be no regularity between the emptying of the two.

The macronucleus is ovoid in outline and appears to be made up of a dense reticulum of chromatin. It lies in the anterior portion of the cell slightly to the right of the center. The single micronucleus is found just anterior to the macronucleus. It is relatively large and spherical and stains very intensely with all of the basic dyes.

Allosphærium palustris is found commonly although never abundantly on the carapace and gill lamellæ of *Orchestia palustris*. In a small number of specimens of *Talorchestia longicornis* a few ciliates of this species were encountered. We have never obtained this species of ciliate from *Orchestia agilis*.

Allosphærium sulcatum gen. nov., sp. nov. (Fig. 1, *B*).—This species is the smallest member of the genus. In length it averages 26 μ, the range being between 24 μ and 32 μ. The most characteristic feature to be noted is the presence of a deep groove or sulcus on the dorsal surface well toward the left margin. This sulcus extends from the level of the cytostome nearly to the posterior end of the organism. It makes a sharp dip to the left near its distal end.

The ventral ciliated surface is somewhat restricted in this species due to the development of the lateral pellicular folds. The right fold encloses approximately one-fifth of the ventral surface while the lapel-like left fold extends over a portion of the endoplasmic sphere. The pellicular folds are continuous around the posterior end in *A. sulcatum*, producing a deep rounded notch at the posterior end of the lapel.

There are only twelve rows of cilia in this species. They are arranged in the same general manner as those of the preceding species. We have never been able to detect the presence of a posterior suture, however, but this may be due to the obscuring effect of the pellicular lapel.

The cytostome is similar in shape and position to that of *A. palustris*. The ciliary membranes are arranged in the same order but the two posterior ones are more attenuated at their proximal ends.

The nuclei and the contractile vacuoles of *A. sulcatum* are similar in relative size and position to those of *A. palustris*.

Allosphærium sulcatum is found regularly but in small numbers on the carapace of *Orchestia agilis* and *Orchestia palustris*.

Allosphærium granulosum gen. nov., sp. nov. (Fig. 1, *C*).—This species is characteristically more rotund than any of the other members of the genus. When viewed from the ventral side its shape is nearly oval and the width of the body is approximately three-fifths that of the length. The smooth dorsal surface is highly vaulted, making this organism quite thick dorso-ventrally. The ciliated portion of the ventral surface is nearly flat. The position and extent of the ventral pellicular folds are like those of *A. sulcatum*, although the left lapel does not extend quite to the endoplasmic sphere.

The ventral surface possesses seventeen rows of cilia originating as

Fig. 1. All figures are composite drawings from living and stained material, drawn from the ventral side. × 1500.

A. Allosphærium palustris gen. nov., sp. nov.
B. Allosphærium sulcatum gen. nov., sp. nov.
C. Allosphærium granulosum gen. nov., sp. nov.
D. Allosphærium caudatum gen. nov., sp. nov.
E. Allosphærium convexa gen. nov., sp. nov.
F. Chilodonella hyalina sp. nov.
G. Chilodonella rotunda sp. nov.
H. Chilodonella longipharynx sp. nov.

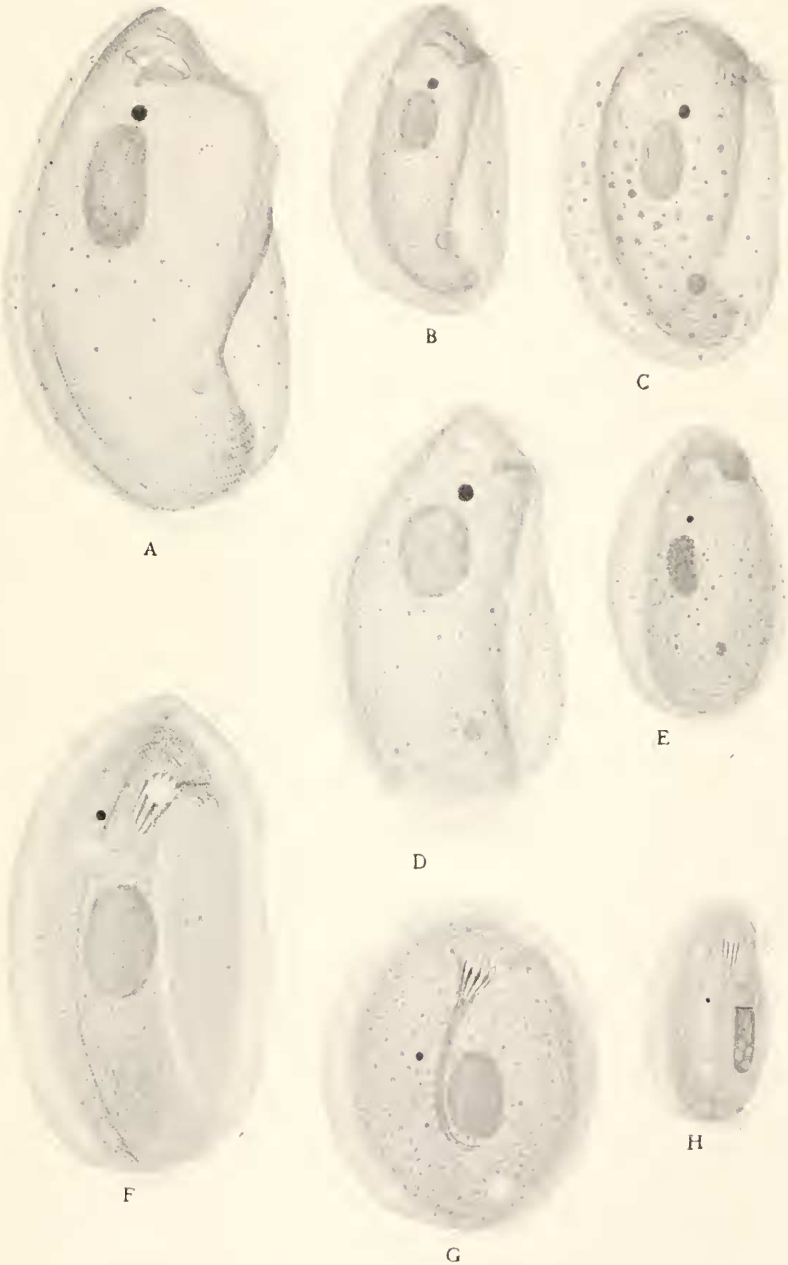

FIGURE 1

in the preceding species. Here again we were unable to detect the presence of a posterior suture probably because of the overfolding lapel.

The cytoplasm of *A. granulosum* is characteristically filled with large granules that render this organism semi-opaque. These granules together with the thickness of the body enable the observer to identify this species with ease.

The nuclei, contractile vacuoles, and cytostomal structure are practically identical with those of *A. sulcatum*.

Allosphærium granulosum averages 38 μ in length, the extremes being between 32 μ and 42 μ. It is found regularly in small numbers on the carapace of *Orchestia agilis* and *Orchestia palustris*.

Allosphærium caudatum gen. nov., sp. nov. (Fig. 1, *D*).—This species resembles *A. palustris* in general body outline. The ventral surface is slightly concave and possesses fourteen rows of cilia. The dorsal surface is weakly arched. A sulcus runs obliquely from near the center of the right margin to the left posterior edge of the organism. Another shallow furrow starts at the level of the cytostome on the left cell border and continues posteriorly nearly the length of the body. It bends somewhat to the left near its posterior extremity. The bend is exactly the reverse of that noted in the case of *A. sulcatum*.

The pellicular fold of the right ventral margin is narrow, as in *A. palustris,* and is of similar extent. The left fold is quite wide, starting at the level of the cytostome and extending to the posterior end. There is no notched appearance in the left fold, the border of which curves slightly to the right at its posterior extremity.

The nuclei resemble those of *A. palustris* except that the micronucleus is somewhat larger and the macronucleus is more spherical than ovoid. There is but one contractile vacuole located well toward the left border of the cell and slightly anterior to the center. This is the only case so far encountered in this genus where there is a single contractile vacuole.

The cytostome and its ciliary apparatus are almost identical with those of *A. sulcatum* and *A. granulosum*.

The most characteristic feature of this species and one that enables the observer to recognize it with ease is the condition of the posterior ectoplasm. Across the posterior end the ectoplasm is drawn out into a shelf. This caudal shelf is very transparent and does not stain with any of the dyes. The posterior cilia, however, are very long and extend under the shelf so that in stained preparations (Heidenhain's hæmatoxylin) the shelf resembles a little fan.

Allosphærium caudatum is found sparsely on the carapace and gill lamellæ of *Orchestia agilis*. We have never encountered it on either

of the other two amphipods used for this study. It averages 41 μ in length, the extremes being 35 μ to 45 μ.

Allosphærium convexa gen. nov., sp. nov. (Fig. 1, *E*).—Because of its regular occurrence and abundance on *Talorchestia longicornis* we have designated this as the type species of the genus in spite of its small size.

As viewed from the ventral side this species is nearly egg-shaped, the narrower end being the anterior. The ventral surface is weakly concave and is supplied with seventeen rows of cilia, arranged in a manner similar to those of the preceding species. Both the right and particularly the left ventral pellicular folds are so narrow that practically the whole ventral surface is exposed. Here the posterior suture is clearly visible. It is an oblique line running anteriorly and to the right from the small notch at the junction of the right and left folds to the endoplasmic sphere.

The cytostome of *A. convexa* is proportionately smaller than any of the other species and is in the form of a nearly circular opening. It is supplied with the usual three ciliary membranes closely resembling those of *A. palustris* as to shape and distribution.

The macronucleus is bean-shaped and lies somewhat to the right near the middle of the body. It is smaller than the macronuclei of any of the preceding species. The micronucleus is minute and occupies a position just anterior to the macronucleus.

There are two contractile vacuoles, the anterior one in the usual position toward the right margin of the cell but the posterior one is found at about the mid-line of the body to the right of the endoplasmic sphere.

Allosphærium convexa averages 29 μ in length. The extremes encountered were from 24 μ to 36 μ. It is found in great abundance on the carapace and gill lamellæ of *Talorchestia longicornis*. We have never encountered it on either *Orchestia agilis* or *Orchestia palustris*.

Chilodonella Strand (Chilodon Ehr.)

Associated with the various species of the genus *Allosphærium* on the carapace of the three amphipods used in this investigation are to be found three species of the genus *Chilodonella*. A large percentage of the hosts examined were found to harbor a few of these ciliates. They are all sufficiently different from previously described species of the genus, as far as we have been able to determine from the literature, to warrant short descriptions. For some time we were in doubt as to the commensal nature of these ciliates, thinking they might represent vagrant free-living forms. A search of the sand and sea weed failed

to disclose any ciliates of like nature, however, and this fact together with the general similarities of the *Chilodonella* and the *Allosphærium* convinced us that we were dealing with forms that normally lived as commensals on the carapace of the amphipod hosts. They live but a short time in sea water when washed free from their host, a characteristic of all species of the genus *Allosphærium*.

Chilodonella hyalina sp. nov. (Fig. 1, *F*).—This species is flattened ventrally and convex dorsally. The dorsal surface bears a longitudinal ridge that extends from near the anterior end and curves toward the right to the posterior end. The lateral margins of the ciliate are extended into a hyaline shelf which reminds one of the brim of a hat. This shelf extends completely around the body except for a short space at the extreme posterior end. It is quite thin and seems to be entirely without visible structure.

The cilia are confined to the ventral surface, as in all members of this genus. They are arranged in twenty-three rows. Nine of these rows originate from the anterior side of a line that extends from the mouth to the left margin of the ciliated part of the ventral surface. These nine rows pass anteriorly and to the right, form an arc above the mouth and then pass down the right margin of the cell to end well over toward the left posterior edge. On the left side of the ciliated area eight rows of cilia originate from the posterior side of the short transverse line. They pass backward and bend slightly to the left. The remaining six rows appear to originate from the region of the mouth. There is a sharp line of demarcation between the eight rows of the left side and the rest of the rows of the ventral surface. These two areas are separated by a naked band that extends from the mouth to the posterior end. In life this band is clearly visible as a light streak separating the ciliated area into two parts. Along the line that extends from the mouth transversely to the left margin arise a row of large, stiff cilia. These cilia beat much more leisurely than do the rest of the body cilia.

The mouth is surrounded by a ring of trichites forming a complete pharyngeal basket. The basket is quite short in this species and extends obliquely into the endoplasm. The trichites taper toward their inner ends and disappear from view among the endoplasmic granules. As in the free-living *Chilodonella*, the pharyngeal basket stains deeply with hæmatoxylin. It is also very conspicuous after the Borrel stain where it takes the acid component (green).

The macronucleus is centrally located. It is oval and is relatively large. The chromatin is evenly dispersed in fine granules without any trace of an "endosome" (see MacDougall, 1925, for a description of

this body in *Chilodon uncinatus*). The micronucleus is located just to the right of the pharyngeal basket.

There are two contractile vacuoles, one just below the micronucleus and the other near the posterior end to the left of the naked band.

Chilodonella hyalina averages 40 μ in length. The extremes are between 36 μ and 47 μ. It is found exclusively on the carapace of *Orchestia agilis*.

In life *Chilodonella hyalina* resembles to a marked degree the various species of the genus *Allosphærium* and without the aid of the oil immersion lens to note the pharyngeal basket it might well be mistaken for a member of that group.

Chilodonella rotunda sp. nov. (Fig. 1, *G*).—The ventral surface of this species is flat but it is strongly arched dorsally. In side view the organism resembles a derby hat with a very narrow brim, while from the ventral surface it appears nearly round. The brim is not hyaline, as it is in the preceding species, but is somewhat granular.

The cilia are arranged much in the same manner as those of *C. hyalina* but the rows are not separated into two groups. There are twenty rows, five of which originate from the anterior side of the transverse oral line.

The pharyngeal basket is quite different from that of *C. hyalina*. It is composed of trichites that show distinct thickenings near their anterior ends. The basket itself is flared out around the mouth. It narrows rapidly into a long gullet supported by the distal ends of the trichites. The gullet extends into the posterior fourth of the body, curving sharply to the left.

The macronucleus is ovoid and is densely and homogeneously granular. It always lies a little back of the center within the curve of the gullet. The micronucleus lies near the center of the cell to the right of the gullet.

There is a single large contractile vacuole very near the midline at the posterior end of the body. The diastolic period is quite long.

Chilodonella rotunda averages 29 μ in length. The extremes were found to be from 27 μ to 34 μ. It is nearly as wide as it is long. So far we have found it only on the carapace of *Orchestia agilis* and never more than two or three specimens on each host infected. It is easily recognized by its general shape, the shape of the pharyngeal basket, and the dark coloration due to the thickness of the granular endoplasm.

Chilodonella longipharynx sp. nov. (Fig. 1, *H*).—This is the smallest representative of the holotrichous ectocommensals found on this group of amphipods. Its reduced size together with its almost crystalline clearness makes it an exceedingly difficult organism to study in life. In

material stained in Heidenhain's hæmatoxylin or the Borrel stain, however, the details of its structure can easily be observed.

The ventral surface is naked except for four rows of rather long cilia. These rows originate anterior to the transverse oral line and describe a semi-circle, up and along the left margin, across the anterior end, down the right margin and thence left across the posterior end. The cilia that originate near the transverse oral line are quite long and relatively thick. As in *C. hyalina* they beat more slowly than do the rest of the cilia along the four rows.

The mouth is surrounded by an extremely long pharyngeal basket which extends nearly to the posterior end of the body. It is made up of straight trichites, which render the basket cone-shaped. In hæmatoxylin preparations the pharyngeal basket is the most conspicuous structure in the cell.

There are two contractile vacuoles both lying to the right of the pharyngeal basket. One is just below the level of the cytostome while the other is in the posterior fifth of the body.

The macronucleus is an elongate oval and is situated along the left margin of the cell. In the majority of ciliates of this species examined there appears a band, very similar to the Kernspalt of numerous hypotrichous forms, extending across the macronucleus. The chromatin on either side of the band is of distinctly different structure, the anterior chromatin being finely granular and faintly staining while the posterior chromatin is made up of a coarse reticulum and stains deeply. The band itself is made up of two parts, a clear plane and an intensely staining plane. This structure is obviously bound up with some stage of development of the ciliate, probably with binary fission. We have found stages where the band was very near to the anterior end of the nucleus and in all gradations of position to about the posterior sixth. Whether the band passes off the end of the nucleus, as it does in a number of hypotrichous forms (see Summers, 1935), or not we cannot say. Although we have hundreds of organisms of this species stained, we have not observed the actual fate of the band. As the band reaches about the halfway point in the nucleus, a deeply-staining sphere is differentiated in the finely granular (reorganized?) portion. This sphere increases in size until its diameter reaches about one-fourth the width of the macronucleus. We cannot say what is the significance or fate of this sphere. We are aware of no other species of *Chilodonella* that shows this band, although it is identical with the one found in *Trochilia* (*Dysteriopsis*) *minuta* described by Roux (1901) and Penard (1922) and seems to be similar to those of a number of species of *Chlamydodon* described by Kahl (1931).

The single micronucleus is very small and is always situated in about the center of the cell on the opposite side of the pharyngeal basket from the macronucleus.

Chilodonella longipharynx averages 19 μ in length, the extremes being from 17 μ to 21 μ. It is found most abundantly on the carapace of *Talorchestia longicornis* and less frequently on *Orchestia palustris*. We have never encountered it on *Orchestia agilis*.

Discussion of Taxonomic Affinities

The genus *Allosphærium* obviously belongs to the sub-class Holotricha Stein (Calkins, 1933) and by virtue of the membranes in the mouth must be included in the order Hymenostomida Hickson (Calkins, 1933). But the allocation to any of the established families of that order offers grave difficulties. In none of the members of the order so far described does there occur a restriction of the cilia to the ventral surface. It seems inescapable, therefore, that a new family should be erected for the reception of the genus *Allosphærium*. We are loath to do this at this time, however, because we feel that the affinities for at least one other form that has previously been described are too close to be disregarded, and as yet the existing description is too fragmentary to warrant an analytical comparison. The form in question is *Lophophorina* Penard 1922. This genus, erected for the reception of a single species (*L. capronata*), resembles *Allosphærium* in size, shape, distribubution of cilia, movements and location on its host (fresh-water *Gammarus*). Indeed we were at first of the opinion that we were dealing with species of the same genus as described by Penard. But when it was seen that our forms all conformed so closely to a set pattern which was different in many important respects from *Lophophorina* we were forced to conclude that we were dealing with a new genus. Penard (1922, p. 96) was unable to locate the mouth but he assumed it to be in the anterior portion of the cell near the long "tentacle." By this it can be readily seen that no diagnosis as to its order is possible without knowledge of so important a structure as the cytostome. Still we feel that there is a possibility that when *Lophophorina* is re-investigated it may be possible to erect a family for the reception of both genera (*Lophophorina* and *Allosphærium*), in which case the family name should be, because of priority, Lophophorinidæ. For the present, therefore, we wish to defer the action of establishing a new family.

The previously described species of the genus *Chilodonella* that resemble most closely those found by us on the amphipods studied are those of Penard (1922). His *Chilodonella* (*Chilodon*) *capucinus* (p. 92) possesses two contractile vacuoles as do our *C. hyalina* and *C.*

longipharynx. It has a spherical macronucleus and the cilia are divided into two areas. There are only ten ciliary rows, however, (five right and five left) as contrasted with the twenty-three rows of *C. hyalina*. The shape of the macronucleus and the ciliation set it apart from *C. longipharynx* while the possession of ten ciliary rows as opposed to twenty for *C. rotunda* together with the difference in contractile vacuole number show it to be distinct from the latter species. *Chilodonella* (*Chilodon*) *granulatus* (Penard, 1922, p. 95) has the same type of ciliation as *C. longipharynx* but there are from five to seven rows. The pharyngeal basket is short and re-curved and the cell possesses but one contractile vacuole. Both *C. capucinus* and *C. granulatus* are ectocommensal on *Asellus* and *Gammarus*.

It should be pointed out that the general shape of the holotrichous ectocommensals of both the amphipods and isopods that have so far been described is singularly well adapted for their environment. They are all small flat forms and possess ventrally placed thigmotactic cilia (*Chilodonella, Trochilia, Allosphærium*). When one considers the forces, mainly in the form of water currents, to which they must be subjected and which would tend to effect their removal from the carapace of their various hosts, it is seen that the flatness of their bodies and the adhesive powers of their ventral cilia are of absolute necessity. Existing under the same conditions, it is perhaps not surprising that representatives of two orders of ciliates exhibit convergence to such a degree as to render them practically indistinguishable one from the other except under extreme magnifications.

Division of Allosphærium convexa gen. nov., sp. nov.

Although we have encountered cases of binary fission in practically all of the species described above, we have been able to trace the process completely in only one form, *Allosphærium convexa*. This species occurs in relatively large numbers and we have had an opportunity to study many hundreds of specimens. Of these a rather high percentage were in some phase of binary fission.

Our observations on the process of division of this minute ciliate are confined mainly to the behavior of the macronuclear chromatin. The cytoplasmic structures and the micronucleus are too small to permit us to follow all of the details of their divisional activity.

The chromatin of the macronucleus appears as a dense reticulum during the resting period (Fig. 2, *A*). It stains intensely with all of the nuclear dyes. With the Feulgen nuclei-acid reaction the reticulum appears to enclose many vacuole-like spaces of varying sizes.

The first sign of fission is to be seen in the peculiar activity of the

central portion of the macronuclear chromatin. A core of chromatin forms in the center of the nucleus, becomes very finely granular, and loses, to some extent, its affinity for basic dyes. A clear zone surrounds

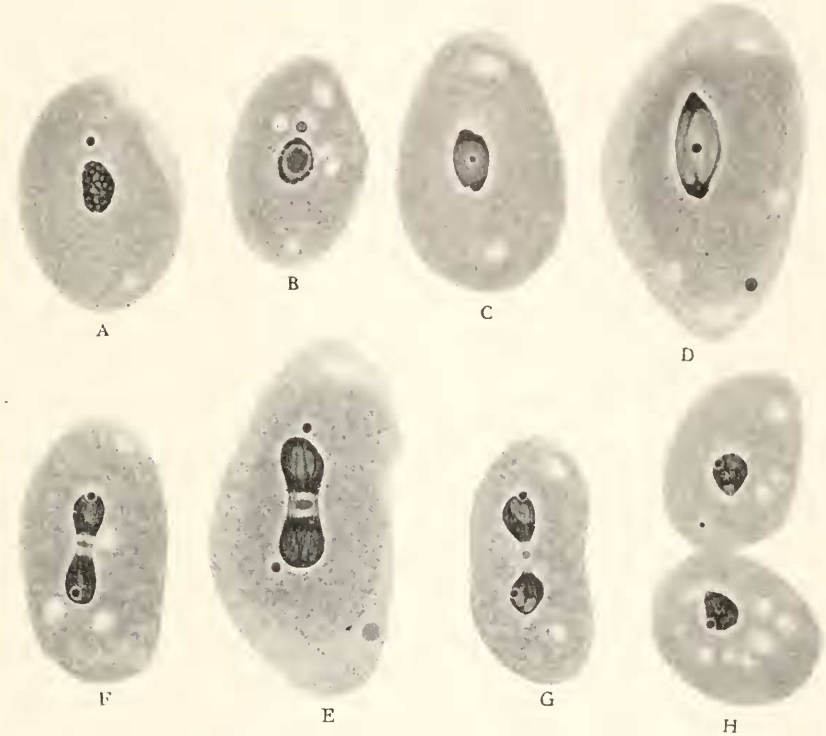

FIG. 2. All figures are of *Allosphærium convexa* during binary fission. The figures represent optical sections of stained organisms drawn with the aid of the camera lucida. × 990.

A. Resting stage. The macronuclear chromatin in the form of a coarse reticulum. Schaudinn-Feulgen.

B. Formation of the central chromatin ball. The shell of reticular chromatin is shown only at the edges in this optical section. Flemming-Heidenhain.

C. Contraction of central ball. Note the concentration of reticular chromatin at poles. Schaudinn-Feulgen.

D. Elongation of macronucleus. Daughter micronuclei below the level of focus. Flemming-Heidenhain.

E. Constriction stage. Most of the reticular chromatin in bands near the division plane. Flemming-Heidenhain.

F. Later stage. The central ball has become loosened and spread out between the future daughter macronuclei. Schaudinn-Feulgen.

G. Separation stage. Schaudinn-Feulgen.

H. Macronuclear division completed. The central ball is cast into the cytoplasm, in this case, of the anterior daughter. Schaudinn-Feulgen.

it, separating it from the shell of unchanged reticular chromatin (Fig. 2, *B*). At this time the activity of the micronucleus is evidenced by an increase in size and by dispersal of its chromatin as fine granules.

The central core of the macronucleus gradually contracts and becomes basophilic, ultimately forming a small sphere in the center of a relatively clear region. In the meantime the reticulate shell clumps into large chromatin masses, with the major portion passing to each pole as the nucleus elongates (Fig. 2, *C*). As elongation proceeds the mid-region, with the exception of the central sphere, becomes clear and nearly free from chromatin. Slightly later the chromatin of the reticulum is found massed at the two poles, connected, however, by numerous fine strands that pass around the clear mid-region (Fig. 2, *D*).

As the macronucleus begins to constrict, the entire chromatin content behaves in an unusual manner. Most of the "active" chromatin forms long, thread-like bodies and migrates toward the division plane, leaving, however, two small polar caps. On either side of the division plane the thread-like bodies form two plates, resembling chromosomes in the anaphase (Figs. 2, *E* and *F*). Their appearance is never regular, however, and we would certainly hesitate to call these bodies macronuclear chromosomes. The chromatin of the central ball loses its compactness and spreads out across the division plane. It is always quite definite and stains clearly after the Feulgen reaction as well as with the basic dyes (Fig. 2, *F*). Further constriction results in a second contraction of this "waste" chromatin to form the residual ball. The chromosome-like bodies of the daughter macronuclei become very irregular and more or less fusion of these bodies with the chromatin of the polar caps ensues (Fig. 2, *G*). As the daughter macronuclei separate, the residual ball of chromatin is cast into the cytoplasm (Fig. 2, *H*) where it decreases in size and is finally absorbed. The chromatin of the daughter macronuclei gradually assumes the structure of the resting reticulum as reorganization proceeds. Plasmotomy proceeds to completion and the daughter ciliates separate.

It may be well to mention the appearance of the endoplasmic sphere during cell fission. There is apparently no division of the sphere since it may be identified in its original position during all division phases. It does not change appreciably in size or staining capacity. The new endoplasmic sphere of the anterior daughter seems to arise *de novo* just anterior to the plane of fission. It may be seen first as a faintly staining area (after Heidenhain's hæmatoxylin) which gradually contracts into a sphere. This would lead one to believe that the endoplasmic sphere is a metaplastid representing some product of metabolism. At least it does not appear to be a self-perpetuating structure.

Discussion of Division

In the light of a number of recent reports the extrusion or elimination of chromatin or chromatin-like materials from the macronucleus during binary fission is not particularly unusual. This phenomenon occurs in a number of holotrichous ciliates, e.g., *Loxocephalus* (Behrend, 1916); *Eupoterion* (MacLennan and Connell, 1931); *Kidderia* (*Conchophthirius*) (Kidder, 1933*a*); *Ancistruma* (Kidder, 1933*b*); *Ichthyophthirius* (Haas, 1933); *Conchophthirius* (Kidder, 1934*b*); *Urocentrum*, *Colpidium*, and *Glaucoma* (Kidder and Diller, 1934). One case that shows striking similarities to that of *Allosphærium convexa* is that described by Rossolimo and Jakimowitsch (1929) in the division of *Myxophyllum* (Raabe, 1934*b*). This ciliate was known until recently as *Conchophthirius steenstrupii* and possesses seven macronuclei in the normal vegetative stage. During division each of the macronuclei, in addition to casting out a part of the chromatin substance, is described as undergoing a type of mitosis. Most of the chromatin takes the form of two groups similar to the chromosomes of a metazoan during the anaphase. There are also two polar caps of chromatin. We have found a number of instances in *Allosphærium convexa* which nearly duplicate the condition in *Myxophyllum*. With such figures at hand one is tempted to draw analogies with true mitosis.

Summary

1. Three species of the amphipod family Orchestiidæ were investigated for ectocommensals. These forms are *Talorchestia longicornis* (Say), *Orchestia agilis* Smith, and *Orchestia palustris* Smith. The material was collected in the vicinity of Woods Hole, Massachusetts.

2. The stomatous, holotrichous ciliates that live as ectocommensals on the amphipods studied belong to two genera: *Allosphærium* gen nov., of which there are five species, and *Childonella* Strand, of which there are three new species.

3. All species of ciliates are well adapted to their environment, being small and flat and possessing ventrally placed thigmotactic cilia.

4. The binary fission of one species (*Allosphærium convexa* gen. nov., sp. nov.) is described. A ball of chomatin is differentiated in the macronucleus and extruded into the cytoplasm just prior to cell fission. Most of the macronuclear chromatin takes the form of long chromosome-like bodies during division, duplicating to a fair degree the condition found in *Myxophyllum*.

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